Genetic susceptibility to Candida infections

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Infections with Candida species

Candida spp., especially Candida albicans, are commensal fungi that reside on the skin, mucosa and gastrointestinal tract of 30 to 50% of healthy individuals at any given time, with everyone being colonized at a certain moment of his/her lifetime (Brown & Netea, 2007). Although C. albicans is not pathogenic under normal host conditions, it can cause severe mucosal or systemic infections when host defense is compromised.

Mucosal infections

Mucosal infections affect the skin and mucous membranes. Common sites for these superficial infections are the mouth, vagina, external ear, skin and nails, of which oral candidiasis is the most common (Odds, 1988). Mucosal infections are usually sporadic, but some patients experience severe and recurrent infections of the skin and oropharyngeal cavities termed chronic mucocutaneous candidiasis (CMC). In addition, most women suffer at least once in their lifetime from vulvovaginal candidiasis, while up to 8% of them have recurrent infections (Sobel, 2007).

Systemic infections

In contrast to mucosal candidiasis which is highly prevalent but does not cause high mortality, systemic infections are life threatening, with mortality rates reaching up to 26–60% (Das et al., 2011). When the organisms enter the blood stream they can invade deep tissues and organs such as brain, heart and kidneys. Considering the number of patients diagnosed each year, Candida has emerged in the recent decades as one of the most important pathogens in sepsis, causing significant morbidity and mortality. Moreover, mortality due to these severe infections has not been significantly changed in the last decade, despite the introduction of potent antifungals such as azoles and echinocandins (Fortún et al., 2012). It is currently believed that only a combination of standard antimycotic treatment with adjuvant immunotherapy may significantly improve the outcome of fungal infections, and both immunological and genetic studies are needed to accomplish the necessary understanding of the pathogenesis of these infections.

Candida albicans host defense

The C. albicans cell wall can be divided into two distinct layers: the inner layer consisting mainly of polysaccharides like chitin, 1,3-β-glucans and 1,6-β-glucans, and the outer layer consisting mainly of proteins that are heavily mannosylated with mannan side-chains. These pathogen-associated molecular patterns (PAMPs) can be recognized by several pathogen recognition receptors (PRRs), such as the Toll-like receptors (TLRs) and C-type lectins (CLRs) on the surface of antigen presenting cells (APCs). TLR2 recognizes phospholipomannans (Jouault et al., 2003), and TLR4 recognizes O-linked mannans (Netea et al.,...
Genetic susceptibility of transcription factors like NF-(MYD)88. Subsequently, a mitogen-activated protein kinase through adaptor proteins like myeloid differentiation factor recognized by TLRs, signals are transduced intracellularly (Gross et al, 2006). Dectin-1 can induce cytokine production (Rogers et al, 2005) and caspase recruitment domain 9 (CARD9) of proinflammatory cytokines. Dectin-1 signals through Syk use different adaptor molecules, which transduce a signal by molecules engage with the receptor. Different types of PRRs 2001) (Fig 1).

When a PRR recognizes its corresponding ligand, adaptor molecules engage with the receptor. Different types of PRRs use different adaptor molecules, which transduce a signal by activating a kinase cascade, in order to induce the transcription of proinflammatory cytokines. Dectin-1 signals through Syk (Gross et al, 2006). Dectin-1 can induce cytokine production independently of other receptors, as well as synergize with TLRs for an optimal stimulation of the cell. When ligands by TLRs, signals are transduced intracellularly through adaptor proteins like myeloid differentiation factor (MYD)88. Subsequently, a mitogen-activated protein kinase (MAPK) response is activated leading to the nuclear translocation of transcription factors like NF-kB and c-Jun, inducing the transcription of cytokines and chemokines (Akira et al, 2006).

Interestingly, depending on the fungal burden and amount of hyphae formation a second MAPK phase, consisting of MKP1 and c-Fos activation, can be initiated, further promoting proinflammatory responses (Moyes et al, 2010).

The recognition of C. albicans by cells of the innate immune system will lead to phagocytosis (Heinsbroek et al, 2008) and killing of the invading pathogen. At the same time, the production of cytokines is induced that on the one hand activate inflammation, and on the other hand engage and direct the adaptive immune response. Activation of the caspase-1 component of the inflammasome, mediated by the intracellular activation of the NOD-like receptor NLRC3, is a central event leading to the processing of pro-IL-1β and pro-IL-18 into their respective bioactive cytokines, directing the induction of Th17 and Th1 responses, respectively (Cheng et al, 2011; Lalor et al, 2011). IFN-γ production by Th1 cells, and IL-17 production by Th17 cells are important characteristics of the Candida-induced immune response (Netea et al, 2008). Inflammasome and Th17 activation is considered to be a central event for the discrimination of colonization versus invasion with C. albicans at the level of the mucosa (Gow et al, 2011).

### General risk factors for Candida infections

C. albicans is an opportunistic fungal pathogen. In healthy individuals, the immune response will usually clear infections, but an immunocompromised immune system causes a significant increase in the risk for Candida infections. Das et al demonstrated that 92% of Candida bloodstream infections are preceded by a course of broad-spectrum antibiotics (Das et al, 2011), which suppress the growth of the normal bacterial flora and eliminates natural antagonism of fungal colonization of the mucosa. There are several other examples in which Candida acts as an opportunistic pathogen. For example, almost all AIDS and oncologic patients with neutropenia suffer from oropharyngeal candidiasis (Grabar et al, 2008; Viscoli et al, 1999). Furthermore, 41% of patients undergoing hematopoietic stem cell transplantation, for which the immune system is destroyed beforehand, suffer from one or more bloodstream infections within the first ten years after transplantation, 4% of which are caused by Candida spp. The crude mortality rate associated with these Candida-infections is 42% (Ortega et al, 2005). Also

### Glossary

| **Autosomal-dominant** | Mode of inheritance in which the presence of only one copy of a gene on one of the 22 autosomal–non-sex chromosomes, will result in the phenotypic expression of that gene. |
| **Candidemia** | The presence of Candida species in the blood. |
| **Candidiasis** | Fungal infection with any of the Candida species. Includes candidemia (in case of systemic infection). |
| **Chronic mucocutaneous candidiasis (CMC)** | An immune disorder characterized by chronic infections with Candida that are limited to mucosal surfaces, skin and nails. |
| **Genetic variation** | Variations of genomes between members of species or between groups of species. Includes SNP (in case it is a common genetic variant), mutation (in case it is a rare genetic variant) and copy-number variation. |
| **Immunodeficiency** | A state in which the immune system’s ability to fight infectious disease is compromised or entirely absent. |
| **Immune paralysis** | A state in which induction of tolerance is due to injection of large amounts of antigen that remains poorly metabolized. |
| **Neutropenia** | An immune disorder characterized by an abnormally low level of neutrophils. |
| **Pathogenesis** | The mechanism by which the disease is caused. |
| **Pathogen recognition receptors (PRRs)** | Proteins expressed by cells of the innate immune system, which recognize pathogen-associated molecular patterns (PAMPs) from microbial pathogens. |
| **Polymorphism** | Having multiple alleles of a gene within a population, usually linked to different phenotypes. |
| **Single nucleotide polymorphism (SNP)** | DNA sequence variation occurring when a single nucleotide in the genome differs between members of a biological species or paired chromosomes in an individual. |
Figure 1. Schematic overview of the anti-\textit{Candida albicans} immune response. When \textit{Candida} is recognized by Toll-like receptors (TLRs) and C-type lectin receptors, the production of cytokines is initiated through activation of transcription factors like NF-κB. IL-1β and IL-18 first need to be cleaved by the NLRP3 inflammasome before they can be secreted. IL-2 is involved in the differentiation of all effector T-cells. The IL-2 receptor is highly expressed on regulatory T-cells (T_{reg}). IL-12 and IL-18 promote the differentiation of T helper 1 (Th1) cells, with IFN-γ being their main product. IL-4 and IL-10 promote the differentiation of Th2 cells, while IL-10 can also suppress Th1 cells. IL-1β, IL-6 and IL-23 drive the development of Th17 cells. DOCK8 is involved in the maintenance of Th17 cells. IL-17 promotes the recruitment of neutrophils, which have tissue protective effects by the production of beta-defensins. Cytokines are recognized by cytokine receptors, which use several adaptor molecules like STAT1, STAT3 and TYK2. PTPN22 is involved in B- and T-cell receptor signaling. Components with mutations and/or genetic variation known to be associated with \textit{Candida} infection are shown in color. APC: antigen presenting cell, BCR: B-cell receptor, CARD9: caspase recruitment domain 9, DC-SIGN: dendritic cell-specific ICAM-grapping non-integrin, MBL: mannose binding lectin, MMR: macrophage mannose receptor, NLRP3: NACHT, LRR and PYD domains-containing protein 3, TCR: T-cell receptor, TLR: Toll-like receptor.
patients with systemic lupus erythematosus (SLE), which are treated with glucocorticoids and other immunosuppressive agents, have an increased risk for invasive fungal infections (IFI), which are predominantly caused by Candida spp. (Fan et al, 2012).

Not only a weakened immune system increases the risk for Candida infections, also the extent to which individuals are colonized with pathogens plays a significant role in the development of candidiasis. Candidiasis typically affects patients with prolonged hospitalization. Fifty-one percent of Candida blood-stream infections is associated with being admitted to the ICU (Das et al, 2011). The mean time of onset of systemic Candida infections is 22 days after hospitalization (Wisplinghoff et al, 2004). Furthermore, when barriers to the outside world are damaged or breached by medical devices or surgery, this creates a portal of entry for pathogens like Candida. The risk of candidemia, 50% underwent recent surgery (Das et al, 2011). Another factor contributing to systemic candidiasis is the fact that Candida spp. can form biofilms on many medical devices like central venous catheters (CVC), contact lenses, intrauterine devices (IUDs) (Donlan & Costerton, 2002) and pacemakers (Glöckner, 2011). Candida can even cause prosthetic joint infections, although they are considered to be rare (Springer & Chatterjee, 2012). Indeed, neonates on the intensive care unit (ICU) with a central line often suffer from infections, with the third most causative pathogen being Candida spp. Fortunately this incidence is decreasing due to the use of anti-fungal prophylaxis (Chitnis et al, 2012).

Genetic risk factors for Candida infections

In spite of the important role played by these risk factors, they do not explain all Candida infections, and only a minority of individuals at risk will eventually develop a fungal infection. It is therefore believed that also genetic factors must play an important role in determining the susceptibility to Candida infections. Indeed, mutations in single genes were found to be responsible for severe Candida infections in several primary immunodeficiencies that display the clinical picture of monogenic disorders. However, these disorders are rare, and in the majority of patients no sole causative genetic factor can be found. In most patients a combination of type polymorphisms and/or environmental factors will determine whether a patient will develop a Candida infection. The genetic susceptibility to more common Candida infections such as RVVC or candidemia is likely polygenic, but the understanding of the genetic factors that determine it is nevertheless crucial for future immunotherapeutic approaches in these patients.

Monogenetic disorders

Several monogenetic disorders have been described in the literature to be associated with an increased susceptibility to fungal infections. Glocker et al described that a homozygous mutation in the CARD9 gene, coding for a protein downstream of Dectin-1, results in an increased susceptibility to both mucosal and invasive Candida infections (Glocker et al, 2009; Lanternier et al, 2012). Disease severity in these patients is likely explained by the fact that CARD9 is also involved in the downstream signaling of several other CLR receptors, such as Dectin-2 and Mincle (Robinson et al, 2009; Saji et al, 2010; Strasser et al, 2012; Yamasaki et al, 2008), implying that CARD9 is a central mediator of anti-Candida host defense.

Another monogenetic disorder that results in an important primary immunodeficiency associated with Candida infections is CMC. Both autosomal recessive and autosomal dominant variants of the disease have been described. Mutations in the CC-domain of STAT1, a signaling molecule downstream of the type I and type II IFN receptor (Darnell et al, 1994), but also IL-23 and IL-12 receptors (as heterodimer with STAT3 or STAT4), have recently been demonstrated to be the main cause of autosomal-dominant CMC (van de Veurdonk et al, 2011), and these findings were confirmed by several other research groups (Depner et al, 2012; Hirata et al, 2012; Liu et al, 2011; Martinez-Martinez et al, 2012; Moreira et al, 2012; Okada et al, 2012; Smeekens et al, 2011). In addition to STAT1 mutations, Puel et al demonstrated the presence of mutations in IL-17RA and IL-17F in some unexplained CMC cases (Puel et al, 2012). In contrast, patients with autosomal recessive autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) not only suffer from CMC, but also experience autoimmune phenomena (Lilic, 2002). APECED has been linked to mutations in the autoimmune regulator (AIRE) gene (Björres et al, 1998) that result in a loss-of-function phenotype, causing the production of neutralizing autoantibodies against important cytokines with antifungal properties such as IL-17E, IL-17F and IL-22 (Puel et al, 2010).

Another monogenetic defect resulting in a primary immunodeficiency syndrome associated with Candida infections of the skin is hyper-IgE syndrome (HIES). HIES was first described as Job’s syndrome and is characterized by high serum IgE levels, eczema, recurrent mucosal infections with C. albicans, and skin and pulmonary infections with Staphylococcus aureus (Davis et al, 1966). There are a number of mutations known to be associated with HIES. Several mutations have been found in STAT3 (Holland et al, 2007; Minegishi et al, 2007), a signaling molecule downstream of the IL-23 receptor, resulting in absent IL-17 production (de Beaucoudrey et al, 2008; Ma et al, 2008; Milner et al, 2008; Sharfe et al, 1997). Other genes which have been associated with HIES include dedicator of cytokinesis (DOCK)8 that codes for a protein involved in Th17 polarization (Engelhardt et al, 2009) and TYK2 (Minegishi et al, 2006), coding for a Janus kinase (JAK) downstream of the IL-12 receptor (Shimoda et al, 2000). All in all, defective Th17 responses underlie both CMC and HIES, two immunodeficiencies associated with severe, chronic, mucosal Candida infections. This emphasizes the importance of the Th17 response in mucosal Candida immunity.

Also mutations in genes coding for cytokines and their receptors have been described to be associated with Candida infections. For example, IL-12Rb1 deficiency has been linked to...
mucocutaneous Candida infections, and these patients also have increased susceptibility for invasive candidiasis (Rodríguez-Gallego et al, 2012). Sharfe et al described a patient with a deletion in the CD25 gene, suffering from esophageal candidiasis. CD25 is the α-subunit of the IL-2 receptor, which is constitutively expressed on T regulatory cells (Sakaguchi et al, 1995). Furthermore, IL-2 is involved in the differentiation of effector T cells. Although Sharfe et al only described a single patient, this again emphasizes the importance of T cells in the anti-Candida host response. A complete overview of monogenetic disorders causing fungal infections is depicted in Table 1 and Fig 1.

Common genetic variants and susceptibility to Candida infections
Despite the presence of primary immunodeficiency syndromes with fungal infections, the vast majority of fungal infections is not present in these individuals, but are common diseases with a polygenic pattern of increased susceptibility. Several studies have been published showing a link between genetic variation and an increased risk for Candida infections, with different genetic pattern being discerned between mucosal and systemic candidiasis. An example of this dichotomy is the role of a Dectin-1 polymorphism for susceptibility to mucosal, but not systemic, candidiasis. We have recently described a family in which its members suffered from recurrent vulvo-vaginal candidiasis (RVVC) and onychomycosis. Their symptoms could result in defective β-glucan recognition and Th17 responses. Interestingly, this polymorphism is present in up to 8% of the Europeans and up to 40% of some sub-Saharan African populations (Ferwerda et al, 2009), being associated with mucosal Candida colonization and treatment in haematopoetic patients (Plantinga et al, 2009), but not with systemic candidiasis (Roseutul et al, 2011).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Mode of inheritance</th>
<th>Phenotype</th>
<th>Disease</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CARD9</td>
<td>Q295X</td>
<td>Autosomal-recessive</td>
<td>Reduced TNF-α production and Th17 cells</td>
<td>CMC</td>
<td>Glocker et al (2009)</td>
</tr>
<tr>
<td></td>
<td>Q289X R101C</td>
<td>Autosomal-recessive</td>
<td>Reduced Th17 responses</td>
<td>Invasive dermatophytic disease</td>
<td>Lanternier et al (2012)</td>
</tr>
<tr>
<td>CD25</td>
<td>Deletion (60–64)</td>
<td>Autosomal recessive</td>
<td>Reduced number of CD4+ cells</td>
<td>Candida esophagitis</td>
<td>Sharfe et al (1997)</td>
</tr>
<tr>
<td>DOCK8</td>
<td>Multiple deletions and point mutations</td>
<td>Autosomal-recessive</td>
<td>Reduced Th17 cells</td>
<td>Hyper IgE syndrome</td>
<td>Engelhardt et al (2009)</td>
</tr>
<tr>
<td>IL-12Rb1</td>
<td>Multiple point mutations</td>
<td>Autosomal-recessive</td>
<td>Low levels of IFN-γ</td>
<td>Mucosal candidiasis</td>
<td>Rodríguez-Gallego et al (2012)</td>
</tr>
<tr>
<td>IL-17RA</td>
<td>Q284X</td>
<td>Autosomal-recessive</td>
<td>Absent IL-6 and GRO-a production</td>
<td>CMC</td>
<td>Puel et al (2011)</td>
</tr>
<tr>
<td>IL-17F</td>
<td>S65L</td>
<td>Autosomal-dominant</td>
<td>Reduced IL-6 and GRO-a production</td>
<td>CMC</td>
<td>Puel et al (2011)</td>
</tr>
<tr>
<td>STAT1</td>
<td>R274W A267V</td>
<td>Autosomal-dominant</td>
<td>Reduced IL-17, IL-22 and IFN-γ production</td>
<td>CMC</td>
<td>van de Veerdonk et al (2011)</td>
</tr>
<tr>
<td>STAT3</td>
<td>Multiple point mutations</td>
<td>Autosomal-dominant</td>
<td>Reduced IL-17 production</td>
<td>HIES</td>
<td>Holland et al (2007)</td>
</tr>
<tr>
<td>TYK2</td>
<td>Deletion (550–553)</td>
<td>Autosomal-recessive</td>
<td>Reduced Th1 and Type I IFN responses</td>
<td>HIES</td>
<td>Minegishi et al (2006)</td>
</tr>
</tbody>
</table>

Genetic variation localized in other PRRs, such as the TLRs, has also been associated with an increased susceptibility to fungal infections. Three single nucleotide polymorphisms (SNPs) in the TLR1 gene have been shown to influence susceptibility to candidemia, presumably mediated by decreased levels of IL-8 and IFN-γ (Plantinga et al, 2012). However, these findings need to be replicated in independent studies, and it is unclear which component of Candida is recognized by TLR1. A similar observation has been made for TLR2 and TLR4, which recognize phospholipomannans and O-linked mannans, respectively. The R753Q TLR2 polymorphism increased the risk for candidemia in one small study through decreased IFN-γ and IL-8 levels (Woehrle et al, 2008), and two SNPs in the TLR4 gene were shown to be a risk factor for candidemia through increased IL-10 production (Van der Graaf et al, 2006), but these observations were not replicated in a larger study of patients (Plantinga et al, 2012). Nahum et al suggested that the L412F TLR3 polymorphism increases the risk for CMC, an effect mediated by decreased IFN-γ production (Nahum et al, 2011). Furthermore, variable number of tandem repeats in MBL2 gene that codes for the soluble PRR MBL has been linked to RVVC in two separate studies (Babula et al, 2003; Giraldo et al, 2007). Finally, length polymorphisms in the NLRP3 gene, coding for the receptor subunit of the NLRP3 inflammasome, can increase the risk for RVVC (Lev-Sagie et al, 2009).

In addition to the first step of pathogen recognition, genetic variation in several cytokines has been linked to an increased risk for Candida infections. Choi et al demonstrated that the −1089T/G, −589C/T and the −33C/T polymorphisms in IL-4 are associated with chronic disseminated candidiasis (Choi et al, 2003). Interestingly, the −589T/C SNP has also been
demonstrated to pose a risk for RVVC (Babula et al, 2005). The −1082A/G polymorphism in the anti-inflammatory cytokine gene IL-10 and the 274INS/DEL polymorphism in IL-12b, are associated with persisting candidemia (Johnson et al, 2012). These data strongly suggest that the balance between pro- and anti-inflammatory cytokines represent an important component of host defense against both mucosal and systemic candidiasis.

The −44C/G polymorphism in DEF1, coding for beta-defensin 1, is correlated with increased Candida carriage (Jurevic et al, 2003). The exact underlying mechanism is unclear, but in general beta-defensins are secreted by neutrophils and epithelial cells and contribute to epithelial immunity. The R620W polymorphism in PTPN22, a protein involved in T-cell and B-cell receptor signaling, was suggested to be associated with an increased risk for CMC. Although the potential mechanism of this association is unclear, but in general beta-defensins are secreted by neutrophils and epithelial cells and contribute to epithelial immunity.

The current body of evidence has provided many new insights into the working mechanism of the anti-Candida immune response. These new insights can pinpoint novel potential targets for immunotherapy. For example, several studies have demonstrated a correlation between decreased IFN-γ levels and an increased risk for systemic Candidiasis (Johnson et al, 2012; Woehrle et al, 2008). A double-blind, randomized, placebo-controlled study is currently being performed using recombinant IFN-γ therapy in sepsis. It would be also very relevant to try and reverse the immunoparalysis (Leentjens et al, 2012). This suggests that IFN-γ is a promising treatment option in sepsis-induced immune paralysis. We are currently investigating the efficacy of recombinant IFN-γ in patients with Candida sepsis.

Despite the significant progress of the last few years for uncovering susceptibility to fungal infections, there are still a significant number of Candida infections for which the environmental and/or genetic risk factors are not yet deciphered. Even more importantly, in spite of current treatment regimens, mortality rates associated with systemic infections are still very high, and in order to improve diagnostic- and treatment options, efforts should be directed towards gaining more insights into the anti-Candida host immune response. This can be achieved in several ways. Discovering novel mutations that underlie monogenetic disorders associated with Candida infections can generate crucial information about a particular gene or protein, and the pathway in which this protein is involved. For example, the use of next generation sequencing and whole exome sequencing to discover STAT1 mutations as a cause of CMC (van de Veerdonk et al, 2011), has also led in the understanding of its role for the generation of Th1 and Th17 responses and the anti-Candida host defense (Smeekens et al, 2011). This discovery can lead to novel approaches to the therapy of CMC, some of them being currently tested.

Of course, the list of existing monogenetic disorders is relatively small, as the majority of Candida cases are likely polygenic and/or multifactorial. In order to investigate this type of disorders other methods will have to be employed such as genome-wide association studies (GWAS), deep sequencing, and systems biology. We have recently used a combination of transcriptional analysis and functional genomics to demonstrate that type I IFNs play an important role in the anti-Candida host defense (Smeekens et al, 2013). Stimulation of circulating leukocytes with C. albicans led to a transcription profile with
overrepresentation of genes from the type I IFN pathway. Subsequently, we showed that polymorphisms in these genes modify Candida-induced cytokine production and influence susceptibility to systemic Candida infections. Furthermore, validation studies showed that type I IFNs skew Candida-induced cytokine responses from Th17 toward Th1, while STAT1-deficient CMC patients display defective expression of genes in the type I IFN pathway. This ‘systems approach’, that integrates the information on anti-Candida host defense from several types of studies, provides information with respect to potential novel anti-Candida immune responses that may represent targets for immunotherapy. It is to be expected that an integration of efforts from immunology, genetics, microbiology and systems biology will represent the novel level of understanding of host defense against fungal (and other) pathogens, improving the outcome of these severe infections.

The authors declare that they have no conflict of interest.

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References


Hospital Database on HIV. (2008) Causes of the first AIDS-defining illness and subsequent survival before and after the advent of combined antiretroviral therapy. HIV Med 9: 246-256


Van der Graaf CAA, Netea MG, Morrè SA, Heijer Den M, Verweij PE, van der Meer JW, Kullberg BJ (2006) Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for Candida bloodstream infection. Eur Cytokine Netw 17: 29-34


